THE EFFECTS OF POTASSIUM CYANIDE, POTASSIUM ARSENITE, AND ETHYL URETHANE ON RESPIRATION IN PELOMYXA CAROLINENSIS[1]

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Introduction

It has been known for some time that cyanide and carbon monoxide inhibit the normal processes of oxidation in living cells. The effect of cyanide on respiration has been studied both on cells of metazoan tissues and on some of the Protozoa. Lund (1918), Gerard and Hyman (1931), and Shoup and Boykin (1931) all found that respiration in Paramecium is not inhibited by cyanide. Lwoff (1934) found that the respiration of *Glaucoma piriformis* in peptone solution was at first reduced as much as 80 per cent by KCN, but later returned to normal or nearly so. Peters (1929) found that M/500 KCN did not inhibit the respiration of *Colpidium colpoda*. Pitts (1932) found that the respiration of *Colpidium campylum* was slightly reduced by cyanide but that this effect was only temporary. Hall (1941), using an improved technique to avoid the loss of cyanide from the test solution, definitely confirmed the fact that *Colpidium campylum* is sensitive to cyanide.

So far as is known, there are no published investigations dealing with the mechanism of respiration in amoeboid organisms. *Pelomyxa carolinensis* Wilson (*Chaos chaos* Schaeffer), a multinucleate rhizopod, is favorable for physiological studies because it is relatively large and can easily be grown in the laboratory.

Material and Methods

The specimens of *Pelomyxa carolinensis* used in these experiments were of the same strain as those used by Belda (1942) and Pace and Belda (1944). They were grown in Hahnert (1932) solution and were fed by adding paramecia to the cultures. Prior to each experiment, however, the pelomyxae were kept for about a week in a culture solution buffered to maintain a hydrogen-ion concentration of pH 6.8 (Pace and Belda, 1944, Table I). Portions of a centrifuged culture of *Paramecium caudatum* were added every second or third day. The pelomyxae grew well under these conditions and usually contained numerous food vacuoles.

The rate of oxygen consumption was measured by means of a Barcroft-Warburg apparatus. Preliminary tests (Pace and Belda, 1944) had shown that there was no measurable difference in the rate of oxygen consumption between pelomyxae tested in flasks which contained 100, 200, or 300 organisms. In the present series of experiments usually 200 specimens were put into each flask; in a few cases 150 or 300 specimens were used.

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A typical experiment was carried out in the following manner: a 0.4 ml. portion of 10 per cent KOH was put into the inset and a 0.3 ml. portion of 3 N HCl into the onset of 3 of the flasks. These 3 flasks were used as controls. Pelomyxae of uniform size were removed from the buffered culture medium with a capillary pipette under a binocular dissecting microscope and washed in 3 separate portions of fresh sterile culture medium. A 5 ml. portion of sterile culture medium containing the proper number of pelomyxae was then put into each of the 3 Warburg flasks.

A 0.4 ml. portion of a KOH–KCN absorption solution [2] was put into the inset of the 3 remaining flasks and a 0.3 ml. portion of 3 N HCl was put into the onset. Pelomyxae were removed from the buffered culture medium and washed in 3 separate portions of fresh culture medium plus either potassium cyanide, potassium arsenite, or ethyl urethane.

The Barcroft-Warburg apparatus included a total of 7 manometers and flasks. Of these, 6 were prepared as above. A 5 ml. portion of sterile culture solution without pelomyxae was put into the remaining flask which was used as a thermobarometer.

The water bath of the apparatus was kept at $25° \pm 0.05°$ C. The shaking mechanism was operated at the rate of 124 complete cycles per minute through an amplitude of 3 cm. After the manometers and flasks had been put into place with the stopcocks open, the shaking mechanism was run for one hour in order to equalize the temperature of the flasks with that of the water bath. All stopcocks were then closed, and manometer readings were recorded at intervals of one hour.

Results

1. The effect of potassium cyanide on respiration.

In order to ascertain the possible effects of cyanide on the structure and activity of Pelomyxa, several dozen specimens were put into Columbia dishes containing buffered culture solution plus different concentrations of KCN. The specimens were observed carefully under the microscope and compared with other specimens kept in culture solution without KCN.

Practically all the food vacuoles disappear in pelomyxae kept for twelve hours or longer in a solution containing 10^{-2} M KCN. In addition there is a reduction in number or size of both the bipyramidal crystals and the cytoplasmic granules, so that the organisms now appear highly transparent. A number of large vacuoles containing clear fluid are produced in the cytoplasm. Large masses of gelated cytoplasm are found occasionally, both in the interior of the organisms and near the tips of the pseudopodia. Only intermittent movement of the plasmasol can be seen. The hyaline layer appears well-defined, and is much thicker than in normal

[2] The KCN and KOH concentrations of the absorption solutions suggested by Krebs (1935) vary with the KCN concentration of the experimental culture fluid as shown:

Molar concentration of KCN in culture solution	Absorbing solution in inner cup (inset)
10^{-2}	10 ml. 2N KCN + 0.2 ml. N KOH
10^{-3}	10 ml. N KCN + 1.0 ml. N KOH
10^{-4}	5 ml. N KCN + 5.0 ml. N KOH
10^{-5}	1 ml. N KCN + 10.0 ml. N KOH

specimens. The surface of the pelomyxae is covered with small protuberances.

After 24 hours in the solution, the pelomyxae have long, thread-like pseudopodia. Additional clear vacuoles make their appearance and movement of the cytoplasm practically ceases. The plasmagel layer appears to be very thin and it is difficult to handle the organisms without breaking the outer protoplasmic layers. If the outer layer is ruptured no new membrane is formed in the region of rupture, and the cytoplasm flows out into the surrounding culture medium. In lower concentrations of KCN, namely, 10^{-3}, 10^{-4}, and 10^{-5} M, similar effects occur, but in progressively less degree.

When KCN was added to the buffered culture solution in the higher concentrations used (10^{-2} and 10^{-3} M), the hydrogen-ion concentration was reduced. HCl was added to restore the hydrogen-ion concentration to the value of pH 6.8.

TABLE I

The effect of potassium cyanide on oxygen consumption in *Pelomyxa carolinensis*. Temperature 25° C.; hydrogen-ion concentration, pH 6.8. In most of the tests, 200 pelomyxae were used in each flask; in a few tests, 150 and 300 were used. Average volume of one million pelomyxae, 32,000 cubic millimeters.

Molar concentration of KCN	Number of tests	Duration of tests	Average O_2 consumption in mm.^{3} per hour per million organisms	Average O_2 consumption in mm.^{3} per hour per mm.^{3} cell substance	Per cent inhibition
0 (Control)	8	3 to 6 hours	9045±595	0.282±0.018	65.4
10^{-5}	8		3132±387	0.098±0.012	
0 (Control)	9	3 to 5 hours	8962±641	0.280±0.020	63.1
10^{-4}	9		3220±302	0.100±0.009	
0 (Control)	7	4 to 5 hours	8718±548	0.272±0.017	67.5
10^{-3}	7		2840±345	0.089±0.010	
0 (Control)	8	3 to 4 hours	9478±567	0.296±0.018	69.1
10^{-2}	8		2930±248	0.092±0.008	

The results of the experiments with cyanide are shown in Table I. This table shows that there is a reduction of 63 to 69 per cent in the rate of oxygen consumption in Pelomyxa when KCN is present in the culture solution in concentrations of 10^{-5}, 10^{-4}, 10^{-3}, and 10^{-2} M. The highest concentration of KCN, however, produced only slightly greater inhibition than the lowest. These results indicate that cellular oxidation in Pelomyxa is regulated principally, but not entirely, by the cytochrome-cytochrome oxidase system.

II. The effect of potassium arsenite on respiration

Lwoff (1934) found that respiration in *Glaucoma piriformis* is only slightly reduced, and under certain conditions actually increased in the presence of KCN, and concluded that some respiratory mechanism other than that of cytochrome-cytochrome oxidase must be present in this organism. In an attempt to ascertain whether this mechanism might involve glutathione, he tested specimens of Glaucoma

with sodium arsenite. This reagent inhibits the activity of glutathione and other compounds containing –SH groups, but presumably does not affect cytochrome oxidase. Lwoff found that in solutions of 5.26×10^{-4} M and 8.7×10^{-4} M sodium arsenite, respiration in Glaucoma was reduced, respectively, by 75–80 and 90 per cent.

Experiments were carried out with Pelomyxa as above, except that solutions of potassium arsenite instead of potassium cyanide were used. Potassium arsenite was added to buffered culture solution to produce concentrations of 5×10^{-3}, 10^{-3}, 5×10^{-4}, and 10^{-4} M. Additional tests were made with higher concentrations of potassium arsenite, but the organisms were killed in less than 1 hour so that no measurements of changes in the rate of oxygen consumption could be made. The results of the experiments are presented in Table II.

TABLE II

The effect of potassium arsenite on oxygen consumption in *Pelomyxa carolinensis*. Temperature, 25° C.; hydrogen-ion concentration, pH 6.8. Two hundred pelomyxae were used in each flask. Average volume of one million pelomyxae, 34,500 cubic millimeters.

Molar concentration of $KAsO_2$	Number of tests	Duration of tests	Average O_2 consumption in mm.^{3} per hour per million organisms	Average O_2 consumption in mm.^{3} per hour per mm.^{3} cell substance	Per cent inhibition
0 (Control)	6	4 to 5 hours	10,365±467	0.300±0.013	16.3
10^{-4}	7		8,680±525	0.252±0.015	
0 (Control)	6	5 hours	10,240±1,140	0.297±0.033	22.0
5×10^{-4}	6		8,000±467	0.231±0.013	
0 (Control)	7	3 hours	9,894±500	0.287±0.014	29.0
10^{-3}	6		7,025±594	0.203±0.017	
0 (Control)	6	3 to 5 hours	9,831±510	0.285±0.015	34.9
5×10^{-3}	6		6,403±506	0.185±0.015	

This table shows that there is a progressive decrease in oxygen consumption with increasing concentrations of $KAsO_2$. The maximum decrease obtained with the highest concentration of $KAsO_2$ which was not lethal to the pelomyxae was 35 per cent.

III. The effect of ethyl urethane on respiration

Some of the mechanisms of cellular oxidation involve the dehydrogenases. These may be inhibited by the urethanes. Lwoff (1934) found that respiration in *Glaucoma piriformis* was reduced by methyl, ethyl, and propyl urethanes. With 1.66 per cent and 2 per cent ethyl urethane the reduction in oxygen consumption was, respectively, 44 and 57–61 per cent.

Experiments were carried out with *Pelomyxa carolinensis* as above, using ethyl urethane in amounts which yielded concentrations of 0.11 M (1 per cent) and 0.17

M (1.5 per cent) after mixture with culture solution. The results are presented in Table III.

This table shows that with 0.11 M and 0.17 M ethyl urethane the rate of respiration in Pelomyxa decreased, respectively, 35.7 and 65.1 per cent.

Table III

The effect of ethyl urethane on oxygen consumption in *Pelomyxa carolinensis*. Temperature, 25° C.; hydrogen-ion concentration, pH 6.8. Two hundred pelomyxae were put into each manometer flask. Average volume of one million organisms, 31,200 cubic millimeters. Duration of each test, 3 hours.

Molar concentration of ethyl urethane	Number of tests	Average O_2 consumption in $mm.^3$ per hour per million organisms	Average O_2 consumption in $mm.^3$ per hour per $mm.^3$ cell substance	Per cent inhibition
0 (Control)	5	10,670±1,240	0.341±0.040	
0.11	6	6,860± 710	0.219±0.023	35.7
0 (Control)	7	9,210±1,100	0.295±0.035	
0.17	6	3,220± 470	0.103±0.015	65.1

Discussion

The results obtained by investigators in earlier tests with cyanide may be erroneous because of rapid loss of HCN from the test solutions. Failure to obtain inhibition of respiration in Paramecium with cyanide has led to the conclusion that the cytochrome-cytochrome oxidase system is not involved in respiration in this organism. However, since Saito and Tamiya (1937) have reported the presence of cytochrome a and c in Paramecium, additional tests, more accurately controlled, should be made.

The fact that Pitts (1932) and Lwoff (1934) obtained only temporary inhibition of respiration in ciliates with KCN, and that Lwoff (1934) obtained inhibition with KCN in peptone solution but not in glucose-Ringer solution, may have been due to loss of cyanide from the test solutions.

The decreased rate of respiration in *Pelomyxa carolinensis* induced by potassium cyanide was maintained as long as the specimens were kept in the solutions, whereas the inhibition brought about by potassium arsenite was only temporary. After eight or nine hours in $KAsO_2$ the rate of respiration had returned nearly to normal; after 15 to 18 hours it was completely normal. These results may indicate that after the supposed inhibition of the glutathione mechanism in Pelomyxa some other respiratory mechanism may begin to function. On the other hand, it may be that there has occurred a gradual conversion of arsenite in the test solution to arsenate.

There is some evidence in the results obtained by Szent-Györgyi and Banga (1933), Korr (1935), and Cohen and Gerard (1937), that arsenite may inhibit the activity not only of glutathione but also of dehydrogenases. If this be true, the results with Pelomyxa indicate that arsenite inhibits the action of dehydrogenases much less than does urethane.

The degree of inhibition of respiration by ethyl urethane in Pelomyxa is approximately equal to that in Glaucoma (Lwoff, 1934), but in approximately equal concentrations of arsenite, respiration in Pelomyxa is inhibited initially by 29 per cent, compared to 75–80 per cent in Glaucoma. If, as supposed, arsenites inhibit the activity of glutathione but not that of respiratory enzymes, it appears that glutathione is much less important in the respiration of Pelomyxa than of Glaucoma.

These results indicate that the respiratory mechanism of *Pelomyxa carolinensis* differs considerably from that of some of the Ciliata. Whether or not the mechanism of respiration of *Pelomyxa carolinensis* resembles that of other free-living Rhizopoda must await further investigation.

Summary

1. In 10^{-5} M KCN respiration in *Pelomyxa carolinensis* is inhibited by 63 per cent. In much higher concentrations of KCN, up to 10^{-2} M, only slightly greater inhibition occurs.

2. Pelomyxae which have been exposed to potassium cyanide (10^{-5} to 10^{-2} M), for 12 to 24 hours, show many changes in protoplasmic structure.

3. In 5×10^{-3} M potassium arsenite the maximum inhibition of respiration in *Pelomyxa carolinensis* is 35 per cent; this effect, however, is only temporary.

4. In 0.17 M (1.5 per cent) ethyl urethane the respiration of *Pelomyxa carolinensis* is inhibited by 65 per cent.

5. Respiration in *Pelomyxa carolinensis* appears to occur chiefly through a cytochrome-cytochrome oxidase system, and partly through a mechanism involving glutathione.

6. The respiratory mechanism of *Pelomyxa carolinensis,* a rhizopod, differs considerably from that of a number of the ciliates.

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